REMARKS

Claim 12 has been amended as suggested to address the 35 USC 112 issue.

Claim 1 has been amended to make it clear that administration of the acetylcholinesterase inhibitor occurs at least at the beginning of the follicular phase as set out at page 8 line 20.

The only remaining issues are whether claims 1, 2, and 12 - 22 meet the non-obviousness requirement of 35 USC 103 over a combination of Walles, Yorke, Trubinkova and Davis and whether claim 22 meets these requirements in view of a combination of these three documents further combined with the newly cited Polinsky. It is submitted that they do. The applicant appreciates the examiner's agreement with her arguments that the present invention is not obvious in view of the previously cited combination of Saiko and Davis.

The present invention involves a central activity involving stimulation of the hypothalmic-pituitary-gonadal axis by administering a centrally-acting acetylcholinesterase inhibitor at the beginning of the follicular phase. In humans this commences about fourteen days prior to the release of an egg. After this administration, the claim requires determining whether a normal follicular response has been obtained and deciding on further administration of said compound based on the results of said determination

A key issue in the examiner's argument is the proper understanding of Walles. As the Examiner points out, "Walles et al. teaches that acetylcholine causes contraction in human and cow follicles" . It appears that this is an immediate reaction. Yorke and Trubinkova teach that this leads to expulsion of the egg. As noted in response to the previous action, this may not in fact be true. However, taking this teaching at face value, to cause contraction of the follicles at the beginning of the follicular phase would therefore cause ejection to occur before the egg develops and so

defeat the whole object of the exercise, which is to cause ovulation of a mature egg.[1]

This combination of references therefore teaches away from the invention as claimed and its requirement to administer any "activating" compound (whatever it is activating) at the beginning of the follicular phase and to monitoring whether a normal follicular response has been obtained

The examiner does not dispute the point made previously that all of Walles requires local direct action on the ovaries and that, even ignoring the timing question, Yorke and Trubinkova only become relevant if the combination of Davis and Welles leads to the expectation that administration of galanthamine, lycoramine or their analogs would result in contraction of the follicles. The Examiner's argument seems to be: Davis teaches certain compounds are acetylcholinesterase inhibitors and it can be assumed that administration of these compounds would result in local activity in the ovaries; the presence of acetylcholinesterase inhibitors is assumed to increase acetylcholine levels; and acetylcholine causes contraction of human and cow follicles (Walles).

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[1] The Applicant advises that , contrary to the assumption made by the examiner, clinical experience tells us that acetylcholine does not in fact cause a human immature follicle to ovulate. There is a natural example of the use of a primarily peripherally-acting cholinesterase inhibitor in sufficient doses to augment muscle contraction in women with myasthenia gravis. Pyridostigmine, a peripherally acting cholinesterase inhibitor is the commonly used drug for treatment of myasthenia gravis. If daily doses of this caused follicles at all stages to ovulate, there would be a continuous supply of corpora lutea. Since menstruation normally occurs about two weeks after ovulation, when the corpus luteum and its hormonal production deteriorate, menstruation would not occur in women with myasthenia who were treated with pyridostigmine. There would be a continuous supply of corpora lutea from post-ovulatory follicles, a continuous supply of estrogen and progesterone, and there would be no menses. They would be in a state of pseudopregnancy. Treated myasthenic women are not usually in a state of pseudopregnancy. Two abstracts (attached) reporting on exacerabations and ameliorations of myasthenia during menstruation make it quite clear that myasthenic women receiving treatment have menstrual cycles. Therefore, increasing acetylcholine to the extent necessary to promote muscle contraction does not cause extrusion of immature eggs in the human female.

In contrast, galantamine, which enters the brain to a much greater extent than pyridostigmine, promoted follicle maturation and did cause pseudopregnancy in dogs, as noted in our prior response. This suggests onereally can drive follicular maturation and ovulation with central cholinergic stimulation, but not with peripheral.

In response to the prior action, it was pointed out that the requirement of the present application was the use of a centrally acting agent (i.e. one that crosses the blood-brain barrier so as to be active in brain) and that Walles was concerned with a purely local stimulation of the ovaries.

The examiner now states "while Davis et al do teach that the compounds must have central activity for treatment of Alzheimer's disease, Davis et al do not teach that the compounds must only have central inhibition of acetylcholinesterase activity or central increase in acetylcholine levels." It is respectfully submitted that this is not the case. Nothing in Davis et al would lead one to expect that the compounds described were suitable for peripheral applications. The emphasis in Davis et al is that its compounds should cross the blood-brain barrier and function primarily in the brain. There is therefore nothing in this teaching that would lead one to expect that the compounds would have any utility as peripheral agents.

Davis 6150354 teaches use of acetyl cholinesterase inhibitors for treatment of Alzheimer's disease. As noted at column 7 lines 50 -54, "To be effective [for treatment of Alzheimer's disease] , a compound must pass the blood brain barrier easily and distribute itself between the central and peripheral nervous systems in such a way that its effect is mainly central, and it must not have significant side effects" This is not a teaching that the compounds used would be compounds to choose for peripheral activity. The examiner's argument thus relies on the assumption that the centrally acting acetyl cholinesterase inhibitors described by Davis will have a peripheral effect. There is no basis for this in the art cited.

Furthermore, noted above, Davis et al teach that side effects are to be avoided. Such side effects stem largely from peripheral activity of cholinesterase inhibitors which are known to include loss of appetite, nausea, vomiting, abdominal pain, and diarrhea (see http://www.answers.com/topic/anticholinesterase-2) and http://en.wikipedia.org/wiki/Acetylcholinesterase_inhibitor) In view of the teaching to

avoid side-effects in Davis et al, and the fact that known side-effects were peripheral, one would not therefore be led to use the compounds described in Davis where the whole emphasis was on central activity for peripheral applications.

It is therefore submitted that the combination of Walles, Yorke, Trubinkova and Davis similarly does not provide any rational basis for administration of a centrally active cholinesterase inhibitor at the beginning of the follicular phase to treat failure of ovulation in humans.

Polinsky adds nothing to this since it simply confirms that rivastigmine is also a centrally acting acetylcholinesterase inhibitor. (40% central inhibition, 10% peripheral inhibition - se abstract). As noted above. Nothing teaches that any member of this class of compounds should be employed to stimulate the H-P-G axis by administration at the beginning of the follicular phase.

For completeness, the applicant also comments on another point made in the action.

The Examiner further states (page 4, third paragraph) "Applicants further argue that it is not clear that muscle stimulation alone could cause extrusion of egg follicles. This argument is also found not persuasive since the prior art references, Trubnikova et al and Yorke et al both teach that muscle contraction is necessary to expel an egg from the follicle."

This argument however ignores the following points:

a. Trubnikova observed "contraction of cells possessing properties characteristic of smooth muscle cells" but the abstract did not state or prove that it was necessary.

b. Yorke, through serial electromicrographs of the egg extrusion, reports that "Constriction of the follicle and extrusion of the egg in the lamprey could be induced by slow changes in the shape of the follicular cells brought about by alterations in the cytoskeleton." (p 906, beginning of third paragraph) The paper ends "The slight forces provided by the changes in the shape of the follicular cells extrude the egg with less stress than would a simultaneous contraction over an essentially enclosed space. Aided by the slippery fluids provided by the fluid of ovulation and by the autolysis of the adhesive cells, the follicular cells slide the egg from the follicle with a slow, steady pressure." (p 906, past some pages of pictures, to p. 911) Yorke mentions that others

have considered that muscle contraction might occur, but shows that it is not involved in lamprey ovulation.

c. Therefore, neither Trubnikova nor Yorke specifically identified smooth muscle cells, and Yorke positively identified the contractions as coming from follicular cells themselves.

d. Espey reviewed "Ovarian Contractility and its Relationship to Ovulation: A Review" (Biology of Reproduction 19, 540-551, 1978, attached). Espey reviews the evidence, including the work by Walles, that acetylcholine is capable of causing ovarian contraction. However, acetylcholine-induced muscle contraction is not necessary for ovulation. "Confirmation of this evidence that ovarian nerves are not essential for ovulation comes from recent demonstrations that ovulation can even occur in perfused ovaries of humans and rabbits ... In fact there were 1.5 times more ovulation points in the perfused rabbit ovaries than in contralateral in vivo control ovaries . . .At the same time, "no association could be noted between the pattern of ovarian contractions and the likelihood of ovulation or the number of ovulations observed" (Hamada et al., 1977). Thus, now there is unequivocal proof that, if contractile activity is involved in the ovulatory process, neuronal stimulation is not necessary." He concludes "Since ovulation and pregnancy can occur in animals with denervated ovaries, it may be concluded that autonomic nerves do not have an essential role in the ovulatory process." (all on page 548)

It is therefore submitted that the present claims meet the requirements of 35 USC 103 and should be allowed. An early action to this effect is respectfully solicited.

Respectfully submitted,

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1: J Neurol Sci. 1998;156(1):107-11.

Exacerbation of myasthenia gravis during the menstrual period.

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BACKGROUND: Myasthenia gravis (MG) is an autoimmune disorder mediated by antiacetylcholine receptor antibodies. It has long been suspected to exacerba during the menstrual period but this has never been adequately documented. SUBJECTS AND METHODS: We questioned 120 female myasthenic patients of differe ages, about their myasthenic symptoms before and during the menstrual period. also evaluated the effect of medications, pain and stress during or before th menstrual period on the exacerbation rate. Exclusion criteria were postmenopa age and incomplete information available in the questionnaire. RESULTS: Forty premenopausal women with generalized disease were included in the study. Twen eight (67%) of the patients reported exacerbation of their myasthenic symptom to 3 days prior to the menstrual period. This exacerbation persisted in 22 of them to the third day of the menstrual period. In nine of the women this clin worsening necessitated an increased intake of medications during the days pri to menstruation. No correlation could be found between the presence of antiacetylcholine receptor antibodies, pain, stress, use of oral contraceptiv or the type of antimyasthenic therapy and the rate of exacerbation before and during the menstrual period. CONCLUSIONS: (1) MG frequently exacerbates befor and during the menstrual period in 67% of MG patients. (2) The rate of exacerbation is unrelated to the presence of stress or pain prior to or durin the menstrual period. (3) Different therapies directed against MG, as well as oral contraceptives do not influence the clinical course. (4) Menstrual exacerbations occur in both seronegative and seropositive patients. (5) These exacerbations may frequently necessitate therapeutic changes.

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Acetylcholinesterase activity and menstrual remissions in myasthenia gravis.

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Menstrually related temporary remissions of myasthenic symptoms are reported occur in 25 to 50% of female patients. Even though this has been attributed t hormonal changes associated with the menstrual cycle the underlying mechanism this hormonal influence has remained elusive. The present study demonstrated cyclical variation in the activity of red cell acetylcholinesterase (EC 3.1.1 enzyme (AChE) with a marked reduction at the time of menstrual remission of

symptoms of myasthenia. These cyclical changes were abolished by thymectomy. appears, therefore, that menstrual remission in myasthenia is at least partly to hormone-induced changes in AChE activity. This process seems to be under t control of the thymus gland.

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Ovarian Contractility and its Relationship to Ovulation: A Review

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INTRODUCTION

During the past decade there has been a renewal of interest in the contention that ovarian contractions are necessary for mammalian ovulation. The century-long deliberation on this controversial issue has been revived by current evidence that: 1) muscle-like cells are present in the vicinity of mature ovarian follicles: 2) contractility is a basic property of ovarian tissue; 3) autonomic nerves extend into the mammalian ovary and 4) neurohumoral agents, prostaglandins and other metabolic substances present in ovulatory tissue can stimulate ovarian contractility. However, despite the extensive new data, it remains unclear whether ovarian contractions have an essential role in the ovulatory process. The problem is no longer a matter of insufficient information as considerable evidence is now available on the topic. What is needed is a comprehensive analysis of the existing data.

This paper critically surveys the current information, in an effort to clarify the functional significance of ovarian contractility in the mechanism of ovulation. The review concentrates on studies that have been carried out during recent years. For a summary of the earlier work on ovarian "smooth muscle," the interested reader is referred to the perceptive report by Kraus (1947). In addition, there are a number of recent papers which discuss various aspects of the subject (O'Shea, 1970; Osvaldo-Decima, 1970; Burden, 1973; Lipner, 1973; Espey, 1974; Walles et al., 1975a; Weiner et al., 1975b).

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Microscopic Observations of Contractile Elements

In considering the morphological composition of the mammalian ovary, it is worthwhile to bear in mind that the ovaries of all mammals have the same basic function, i.e., to produce ova and secrete steroid hormones. Thus, one would expect to find analogous cytological components in the ovaries of different mammalian species. Also, it is likely that the mechanism of ovulation in different species is carried out by similar metabolic processes. Therefore, this review is prepared under the opinion that, if ovarian contractions have a fundamental role in ovulation, then the contractile properties of different mammalian ovaries should be comparable.

Electron microscopy of myoid-like cells. By electron microscopy, ovarian "smooth muscle" has been reported in the rat (O'Shea, 1970; Osvaldo-Decima, 1970), monkey (Osvaldo-Decima, 1970), mouse (Fumagalli et al., 1971), cat (Fumagalli et al., 1971), rabbit (Fumagalli et al., 1971; Okamura et al., 1972; Bjersing and Cajander, 1974), sheep (O'Shea, 1971), human (Okamura et al., 1972), guinea pig (Burden, 1972), hamster (McReynolds et al., 1973), gerbil (McReynolds et al., 1973) and cow (Walles et al., 1975a). The myoid-like tissue in the ovaries of these assorted species has been morphologically described as fusiform cells which contain bundles of thin filaments that extend parallel to the long axis of the

cytoplasm. The diameter of these microfilaments is approximately 60-80 Å. Electron dense bodies and glycogen particles are sometimes located in the vicinity of the filaments. A basement membrane of homogenous thickness is sometimes present along the outer surface of the plasma membrane of the ovarian "smooth muscle" cells and numerous pinocytotic vesicles are distributed along the inner surface of their plasma membrane. Occasionally, short segments of tight junctions can be observed between opposing cell membranes.

The hilar and medullary regions of the mammalian ovary contain obvious smooth muscle; however, there are less consistent accounts of the distribution of "smooth muscle" in cortical and follicular tissue. Burden (1972) has reported that 50% of the cells in the theca externa of the cat, guinea pig and rabbit are "smooth muscle", but, he states that "the apical region of the protruding follicle is devoid of muscle." Bjersing and Cajander (1974) agree that thecal "smooth muscle" cells are common, however, they are at variance with Burden in reporting that myoid cells extend into the apex of rabbit follicles. Also, McReynolds et al. (1973) noted "as many as 5-6 cell layers of smooth muscle" throughout the thecae in preovulatory follicles of the hamster and gerbil. Similarly, O'Shea (1971) has observed that "many of the cells in the theca externa of ovarian follicles in the sheep have the essential ultrastructural features normally associated with smooth muscle." However, in a later paper, O'Shea (1973) modifies his initial position by stating that "fully differentiated smooth muscle cells were seldom present in the theca externa" of the sheep ovary. Furthermore, he found that the theca externa of ovarian follicles in the rat contained mostly fibroblasts (O'Shea, 1970). Likewise, Okamura et al. (1972) noted that "smooth muscle" cells in the theca externa of the rabbit "are rare, requiring extensive and careful search." In addition, Okamura et al. (1972) found it "difficult to identify typical smooth muscle cells in the cat ovary", yet, such cells "are rather abundent in the theca externa of the human ovarian follicle." Finally, during my 12 years of study on the ultrastructure of rabbit follicles, I have rarely observed "smooth muscle" cells in either the apex or the base of mature follicles nor have I seen any appreciable number of myoid cells in the parafollicular stroma.

Comparison between smooth muscle cells and fibroblasts. It is doubtful that the incongruity in the data on the distribution of follicular "smooth muscle" can be attributed entirely to species variation, because some of the cited investigators studied several different species. It is more probable that the disparity is due, at least in part, to the fact that separate investigators have not used corresponding sets of criteria to identify smooth muscle cells. Furthermore, from the various descriptions that are given in the literature, it appears that *too many researchers have relied too much on the identification of cytoplasmic filaments* as the single most important morphological evidence for smooth muscle. In such cases, the investigators have overlooked the fact that filamentous structures are present in a wide variety of animal and plant cells. In fact, such filaments "are presumed to be present in all cells that have the potential to move" (Wessels et al., 1971). (The filaments are thought to be "contractile machinery" for locomotory function during cell migration.)

Fibroblasts, which are the principal cellular component of all thecal tissues, are mobile cells. Therefore, it is not surprising that their cytoplasm usually contains microfilaments (Haust and More, 1967; Wessels et al., 1971; Gabbiani et al., 1973). There is "a thin sheet of microfilaments which lie inside the plasma membrane of fibroblastic cells and most of the filaments run parallel to the long axis of the cells" (Wessels et al., 1971). Thus, in any microscopic evaluation of the cellular composition of the ovary, precautions should be taken to avoid the arbitrary classification of *any* cell with microfilaments as a smooth muscle cell. The need for more discrimination becomes evident when one takes into account O'Shea's (1973) informative description of the theca externa of the sheep follicle, in reference to which he points out that "a high proportion of thecal cells possess filaments at least in localized areas of their cytoplasm, although the number of filaments varies considerably from cell to cell." Even more importantly, O'Shea (1973) has made the discerning observation that, although some micrographs of the theca externa may present profiles of what appear to be predominantly myoid structures, such pictures "were in fact off-centre, or grazing sections of cells containing localized bundles of myofilaments." In other words, the usual, two-dimensional view of thecal cells may sometimes display a striking number of microfilaments within certain cells, but the ultrathin sections happen to reveal only that portion of the fibroblast where there is a high concentration of filaments. Therefore, in searching any tissue for smooth muscle cells, it is indeed erroneous to classify a cell as myoid simply upon the detection of cytoplasmic filaments.

In conjunction with their function in cell

locomotion, cytoplasmic filaments endow fibroblasts with contractile properties (Gabbiani et al., 1973; Izzard and Izzard, 1975). Therefore, since these microfilaments are *contractile* protein, it is not surprising that they are composed of actin and myosin elements (Huxley, 1973; Lazarides and Weber, 1974; Anderson, 1975; Bray and Thomas, 1976). This biochemical characteristic of fibroblasts probably explains why several investigators (Burden, 1973; Kapinus and Rukosuev, 1975; Amsterdam et al., 1977; Lindner et al., 1977) have been able to identify myoid proteins in the cells (fibroblasts?) of the theca externa of mature follicles.

It is also important to note that thecal cells are especially mobile during ovulation, when the follicle ruptures and the fibroblasts begin to proliferate into the lutein granulosa to lay down new collagen and supporting structure for the developing corpus luteum (Espey, unpublished observation). Therefore, during this phase of greater cellular mobility, the number of cytoplasmic filaments might be expected to increase within the follicular fibroblasts. (This conception deserves further investigation in the future.)

Furthermore, it is now recognized that there are certain histologic situations during which fibroblasts become more contractile than usual. "Under such conditions, fibroblasts develop new morphologic, biochemical, and pharmacological characteristics similar to smooth muscle" (Gabbiani et al., 1973). The newly differentiated cell, the "myofibroblast," can influence the evolution of various pathologic processes such as wound healing and the contraction of granulation tissue. Therefore, within the traumatic disruption of tissue during ovulation, it might also be that some thecal fibroblasts develop myoid features, as they begin to migrate through the hemorrhagic blood point and go about healing the damaged tissue at the apex of a ruptured follicle. In addition, the ovulatory surge in ovarian steroidogenesis may have some effect on the formation of contractile elements in thecal fibroblasts, because Wessels et al. (1971) have pointed out that estrogen influences the formation of microfilaments within cells.

Criteria for characterizing myofibroblasts. It is now obvious that there is a need for greater precaution and precision in the identification of contractile elements and the classification of myoid cells in ovarian tissue. To help distinguish myofibroblasts from normal fibroblasts in peri- and parafollicular tissue, researchers are encouraged to refer to Gabbiani's et al. (1973) criteria for characterizing myofibroblasts:

1) "The *fibrillar system* within the cytoplasm (of myofibroblasts) consists, not of a few fibrils as seen in normal fibroblasts, but bundles of filaments (measuring 40-80 Å in diameter), which are usually arranged parallel to the long axis of the cell.... Such fibrillar structures occupy a large part of the cytoplasm, . . .
2) "The *nuclei* consistently show multiple indentations or deep folds, an appearance quite unlike that of normal fibroblasts, but very similar to that of smooth muscle and other cells undergoing contraction. . . .
3) "Numerous *intercellular connections* are present between myofibroblasts; their structure identifies them as maculae adherentes . . .
4) "(A *basal lamina* usually covers part of the cell surface as a well-defined layer.)"

By applying the above criteria to the data given in the various reports on the ultrastructure of follicular tissue, it can be concluded that fibroblasts are the prevalant cell type in the thecal tissue which surrounds Graafian follicles. Myofibroblasts may occasionally appear in this tissue, but there is no convincing evidence that typical smooth muscle cells exist in any significant number in the vicinity of mature follicles. It is true that intercellular connections and basal lamina can be seen occasionally in association with thecal cells, but the respective cells rarely display the nuclear deformations which Gabbiani et al. (1973) have described as typical of myoid cells. In the literature on ovarian smooth muscle, only O'Shea (1970) has mentioned that thecal fibroblasts "may show the irregularities which characterize the nuclei of contracted smooth muscle cells." At the same time, he points out that the nuclei of thecal cells are *usually* regular in outline. Although Walles et al. (1975a) have stated that thecal cells in the cow ovary have "elongated, often irregular nuclei," they do not clarify what they mean by "irregular" and their micrographs show only smooth-surfaced nuclei within thecal cells, which appear to be typical fibroblasts.

Myoid cells in corpora lutea. This section of the critique would not be complete without mentioning that corpora lutea apparently have more myoid features than do mature follicles (O'Shea, 1970; Fumagalli et al., 1971; Okamura et al., 1972; McReynolds et al., 1973; Kapinus and Rukosuev, 1975). O'Shea (1970) has reported that

there is "a well-defined coat of mature smooth muscle cells" surrounding the corpus luteum of the rat. This observation raises the question of what the functional significance is of the smooth muscle tissue in the corpus luteum. O'Shea (1970) is of the opinion that "it seems unlikely that smooth muscle cells around the borders of corpora lutea serve any useful function."

Summary. It appears that typical smooth muscle tissue is confined to the hilar and medullary regions of the mammalian ovary. The numerous reports of similar tissue in peri- and parafollicular areas are probably due to the overenthusiastic identification of "smooth muscle" cells simply on the observation of cytoplasmic microfilaments, without realizing that these structures are also a normal component of thecal fibroblasts. At the time of ovulation and especially during luteinization, there appears to be some differentiation of the thecal fibroblasts into myofibroblasts. Such myoid-like cells may facilitate wound healing and the removal of granulation tissue in ruptured follicles.

Mechanical Measurements of Contractility

More than fifty years ago, Guttmacher and Guttmacher (1921) demonstrated contractility to sow Graafian follicles. They inserted strips of the follicle wall into a tension recording apparatus and exposed the tissue to solutions of physostigmine, barium chloride and hydrochloric acid. These "reagents" induced sustained, nonrhythmical contractions. The tissue showed no evidence of fatigue after extended periods of contraction. Relaxation could be induced only by alkaline solutions.

When an attempt was made to repeat the Guttmacher's classical experiment, no response could be obtained with solutions of physostigmine or barium chloride that had been adjusted to physiological pH (Espey, 1964). On the other hand, hydrochloric acid stimulated substantial contractions when the solutions were adjusted to an acid pH of 3.0 or less. This "contractility" could be reversed by the addition of alkaline reagents. In subsequent studies (Espey, 1967), it became obvious that acid-induced contractions of the follicle are the result of changes in the length of the collagen fibrils in thecal tissue. Therefore, although there is no record of the actual pH of the solutions which the Guttmachers used, the contracitility which they measured was probably due, at least in part, to acid-induced contractions of follicular collagen. Be that as it may, their work has served as a valuable stimulus for recent experiments which have provided clear evidence of contractile activity in mammalian ovaries.

Measurements with force-displacement transducers. Recent studies of ovarian contracility have demonstrated *in vivo* and *in vitro* responses that are more typical of smooth muscle action. Contractility has been reported in the ovaries of the cat (Rocereto et al., 1969), rabbit (Virutamasen et al., 1972; de la Cruz et al., 1976; Weiner et al., 1977), human (Palti and Freund, 1972; Coutinho et al., 1974; Diaz-Infante et al., 1974; Maia et al., 1975; Gimeno et al., 1976), monkey (Virutamasen et al., 1973), sheep (O'Shea and Phillips, 1974), cow (Walles et al., 1975a, 1975b, 1976), guinea pig (Maia et al., 1975) and rat (Roca et al., 1976).

Spontaneous contractions were "detected more frequently *in vivo* than *in vitro*" (Virutamasen et al., 1973). It was not possible to identify the histological origin of the *in vivo* contractions because of the gross nature of the recording technique. However, the *in vitro* studies suggest that rhythmical contractions originate primarily from the hilar and medullary portions of the ovary (Virutamasen et al., 1973; Coutinho et al., 1974; Walles et al., 1975b; Maia et al., 1975; de la Cruz et al., 1976). In addition, rhythmical contractions may occur in the ovarian stroma (Maia et al., 1975; Gimeno et al., 1976; Roca et al., 1976) and in sections of tissue taken from the base of mature follicles (O'Shea and Phillips, 1974; Walles et al., 1975b), however, it is not clear whether the latter source of tissue had been carefully excised in a manner which rendered it devoid of medullary smooth muscle.

When strips of tissue were taken from the apexes of mature follicles, only tonic, dose-dependent contractions could be induced, rather than rhythmical contractions (Walles et al., 1974; Okamura et al., 1975; Owman et al., 1975; Walles et al., 1975b; Gimeno et al., 1976; Walles et al., 1976). This limited response raises further question about the accuracy of the microscopic evidence that the theca folliculi contains "smooth muscle" cells, because a number of the ultrastructural studies concluded that the theca externa contained abundant myoid tissue and, if this were actually the case, then the compact tissue in the apical portion of the follicle wall should manifest even greater contractility than the ovarian stroma, which has comparatively few "smooth muscle" cells.

Also, there is inconsistent information as to whether preovulatory or postovulatory tissue displays greater contractility. Roca et al. (1976)

observed spontaneous activity "at all stages of the estrous cycle" of the rat, but they found it difficult to make a quantitative analysis of their data because of the irregularity of the contractions. However, O'Shea and Phillips (1974) have reported that in the ewe "the strongest contractions recorded were in large luteinized follicles." In contrast, de la Cruz et al. (1976) found ovarian contractility to be "more prominent during the preovulatory than the postovulatory phase" in the rabbit. Similarly, Virutamasen et al. (1973) noted more contractility "during the follicular phase of the cycle" of monkeys. They concluded that the corpus luteum was noncontractile, but their study included only *one* lutein ovary. Thus, it is not possible to decipher a consistent pattern of contractility in relation to the time of ovulation. Therefore, the relevance of ovarian contractility to the mechanism of ovulation remains uncertain.

Fluctuations in intraovarian pressure. In addition to the measurement of ovarian contractility by force-displacement transducers, it is possible to measure the contractility indirectly by a pressure transducer applied in a manner that will provide a continuous record of the hydrostatic pressure within the ovary. By this method, we (Espey and Lipner, 1963) first measured rhythmic changes in the antral pressure of mature follicles of rabbits. The average pressure within the follicle was approximately 15 mm Hg. This pressure occasionally underwent rhythmic deviations of 5-10 mm Hg at intervals of 2-5 cycle/min. These measurable "contractions" were not a prerequisite for ovulation; in fact, most of the time there was no evidence of contractile activity during ovulation. These observations were confirmed by Rondell (1964).

More recently, using modified techniques, other investigators have recorded rhythmic changes in intraovarian pressure in the human (Coutinho et al., 1974; Owman et al., 1975) and rabbit (Virutamasen et al., 1976; Wright et al., 1976). Coutinho et al. (1974) recorded intraovarian pressure by inserting a balloon-tipped catheter into the ovarian mass and connecting it to a pressure transducer. From their brief experiment, Coutinho et al. concluded that an increase "in intraovarian pressure takes place immediately following an intravenous injection of human chorionic gonadotropin (hCG) or LH," or shortly after the administration of luteinizing hormone releasing hormone (LHRH). This response lasted only a few minutes. Unfortunately, their experimental procedure did not include a simultaneous measurement of arterial hydrostatic pressure and therefore, it is not possible to determine whether the changes they recorded were due to hormonal stimulation of ovarian contractility or due to a transient increase in systemic pressure following the hormone injections. Our study (Espey and Lipner, 1963) revealed that intrafollicular pressure is dependent upon and directly proportional to arterial hydrostatic pressure and therefore, the intraovarian reaction observed by Coutinho et al. (1974) may have been related to fluctuations in arterial pressure.

Virutamasen et al. (1976) used a technique similar to Coutinho et al. (1974) to record intraovarian pressure in the rabbit. In control experiments, they measured a relatively steady pressure of approximately 10 mm Hg. A distinct increase in this pressure occurred immediately after the injection of norepinephrine (NE), prostaglandin $F_2\alpha$ ($PGF_2\alpha$) or oxytocin (OT), but (as in the above experiment by Coutinho et al.) they neglected to determine whether these pressure changes were associated with elevations in arterial hydrostatic pressure. In contrast to the immediate increase in "contractility" which Coutinho et al. (1974) recorded following the injection of hCG, Virutamasen et al. (1976) did not detect any increase until 7-8 h after hCG. They inferred that there is a further increase in "contractility" near the time of ovulation. However, instead of observing rhythmic changes in intraovarian pressure, their records show bursts of erratic oscillations in pressure and these fluctuations occurred at unpredictable intervals during the ovulatory process. Thus, their data are difficult to interpret (e.g., see Fig. 6-10, Virutamasen et al., 1976). Particularly disconcerting is the fact that the erratic increases in "contractility" which they reported did not always coincide with the time of ovulation (see Fig. 9, Virutamasen et al., 1976).

Using a different technique to record intraovarian pressure, Wright et al. (1976) implanted a sensitive force transducer into the medulla of the rabbit ovary. Their recordings (see Fig. 3A and 3B, Wright et al., 1976) show considerable irregularity in "contractile activity" from one day to the next and therefore, it is also difficult to derive a substantial conclusion from their results. Be that as it is, they surmised that ovarian contractility increases at approximately 11 h post-hCG, which is close to (or shortly after) the usual time of ovulation in the rabbit.

In another experiment, *in vitro*, Owman et al. (1975) reported slight elevations in antral pressure in the human follicle after the addition of NE to the organ bath. Their data suggest that catecholamines

can stimulate myoid elements within the follicle wall in some manner that induces an increase in intrafollicular pressure (and presumably stresses the follicle). Although these investigators did not provide information on the duration of the induced pressure increase, it is doubtful that the response would have lasted any appreciable period of time in normal tissue *in vivo*, because artificially induced elevations in intrafollicular pressure are rapidly nullified by the transudation of fluid out of the antral cavity and into the vascular compartment (Espey and Lipner, 1963; Rondell, 1964). Therefore, the theoretical contraction of the follicle wall by catecholamine stimulation would not be expected to induce a sustained elevation in intrafollicular pressure.

Thus, the measurement of intraovarian pressure is a difficult experimental procedure which provides only limited information about the cause and effect of the periodic fluctuations in the hydrostatic pressure in ovarian tissue. These rhythmic changes in pressure could be the result of smooth muscle spasms which compress the ovarian blood supply. Such spasms might originate in the mesovarian ligament and adjacent hilar portion of the ovary. It is also possible that intraovarian pressure could fluctuate as a result of spasms in the tubo-uterine vasculature, because vessels of this origin anastomose with ovarian arteries (Weiner et al., 1975a). In considering this possibility, it may be relevant that Markee (1932) has noted that the uterine vasculature sometimes undergoes rhythmic spasms at 15-20 second intervals, depending on the "hormonal" condition of the tissue. Since prostaglandin $F_{2}\alpha$ stimulates the contraction of ovarian arterial smooth muscle (Ford et al., 1977) and since this compound increases in the ovary during ovulation (LeMaire et al., 1973), prostaglandin $F_{2}\alpha$ could be responsible for preovulatory spasms in the ovarian arteries.

Summary. Ovarian contractility has been demonstrated in a variety of species of mammals. The greatest contractility exists in tissue near the ovarian hilus and the least occurs in the follicle apex. The contractile force of this "myoid" tissue may cause minor fluctuations in introvarian pressure. This existing information does not allow a definite conclusion as to whether preovulatory or postovulatory tissue is more contractile.

Neuronal Influence on Contractility

Since the mammalian ovary is stimulated by both the sympathetic and parasympathetic nervous systems (for a review of this subject, see Bahr et al., 1974), it is plausible that ovarian contractility is affected by the action of nerves. Ovarian innervation has been studied in the human, monkey, sheep, dog, cat, rabbit, guinea pig, rat, mouse and cow (Jacobowitz and Wallach, 1967; Bahr et al., 1974; Walles et al., 1974, 1975a, 1976, 1977a, 1977b; Owman et al., 1975; Weiner et al., 1975b; Virutamasen et al., 1976). The sympathetic nerves originate from the lower thoracic region of the spinal cord and pass through the celiac plexus and ovarian ganglia before entering the hilar region of the ovary. The parasympathetic fibers, which are probably of vagal origin, also enter the hilus along with the ovarian blood vessels.

Distribution of nerves in the ovary. The autonomic nerves converge, for the most part, on the ovarian vasculature (Bahr et al., 1974), especially around vessels in the hilum and medulla (Fink and Schofield, 1970; O'Shea, 1970). However, a portion of the nerves pass to the cortex of the ovary and form a plexus around the Graafian follicles (Fink and Schofield, 1970). The nerves are apparently in contact with both developing and mature follicles, but the reported amounts of follicular innervation range from extensive (Walles et al., 1975a, 1976) to nonexistant (McReynolds et al., 1973). Within the follicle, nerves can be found in both thecal layers, but not inside the granulosa (see Bahr et al., 1974). In addition to making contact with the thecal vasculature, some of the nerves may terminate on myofibroblasts (Burden, 1972; Walles et al., 1974, 1975a, 1976, 1977a, 1977b).

Adrenergic and cholinergic receptors. The sympathetic and parasympathetic nerves to the ovary apparently terminate in proximity to effector cells that have receptors which respond to adrenergic and cholinergic agents. Since these effectors are considered to be myoid cells, various adrenergic and cholinergic agents should influence ovarian contractility. To examine this idea, there have been numerous studies on the effects of neurohumoral agents and related compounds on ovarian contractility. However, before summarizing the results of these experiments, it is helpful to begin with a review of the basic characteristics of adrenergic and cholinergic receptors.

The most common agonists (stimulatory agents) of adrenergic receptors are epinephrine, norepinephrine, phenylephrine and isoproterenol. Within a specific tissue, the relative potency of each adrenergic agent is determined by the type of

TABLE I. Effect of adrenergic agents on contractility.

Agent	Effect	Tissue[a]	Mammal	Reference
Alpha Agonist				
NE, E	+	ovary	cat	Rocereto et al., 1969
NE	+	ovary	rabbit	Virutamasen et al., 1972
NE	+	ovary	monkey	Virutamasen et al., 1973
NE, PE	±	ovary	guinea pig	Coutinho et al., 1974
NE, PE	+	ovary	rabbit	Coutinho et al., 1974
NE	+	ovary	human	Okamura et al., 1974
NE, E, PE	+	follicle	sheep	O'Shea and Phillips, 1974
NE	+	ovary	monkey	Diaz-Infante et al., 1975
NE	+	ovary	cat, human	Diaz-Infante et al., 1975
NE, E	+	ovary	guinea pig	Walles et al., 1974
NE, E	+	ovary	cow	Walles et al., 1974
NE, E, PE	+	follicle	human	Owman et al., 1975
NE, TYR	+	follicle	cow	Walles et al., 1975a
NE, E, PE	+	follicle	cow	Walles et al., 1975b
PE, IM	+	ovary	guinea pig	Maia et al., 1975
NE, E, PE	+	follicle	human	Gimeno et al., 1976
NE	+	ovary	rabbit	Weiner et al., 1977
Beta Agonist				
ISO	−	ovary	cat	Rocereto et al., 1969
ISO	−	ovary	rabbit	Virutamasen et al., 1972
ISO	−	ovary	monkey	Virutamasen et al., 1973
IOS	±	ovary	human	Okamura et al., 1974
ISO	−	follicle	sheep	O'Shea and Phillips, 1974
IPA	−	ovary	guinea pig	Walles et al., 1974
IPA	−	ovary	cat	Walles et al., 1974
IPA, TER	−	follicle	human	Owman et al., 1975
ISO, TER	−	follicle	cow	Walles et al., 1975b
ISO	−	ovary	rabbit	Weiner et al., 1977
Alpha Antagonist				
POB	−	ovary	cat	Rocereto et al., 1969
POB	−	ovary	rabbit	Virutamasen et al., 1972
POB	±	ovary	human	Okamura et al., 1974
POA, TOL	−	follicle	sheep	O'Shea and Phillips, 1974
POB	−	ovary	guinea pig	Walles et al., 1974
POB	−	ovary	cat	Walles et al., 1974
POB, DB,	−	follicle	human	Owman et al., 1975
POB, PIP	−	follicle	cow	Walles et al., 1975b
POA	−	follicle	human	Gimeno et al., 1976
POB	−	ovary	rabbit	Weiner et al., 1977
Beta Antagonist				
PRO	+	ovary	cat	Rocereto et al., 1969
PRO	+	ovary	rabbit	Virutamasen et al., 1972
PRO	+	ovary	guinea pig	Coutinho et al., 1974
PRO	+	follicle	sheep	O'Shea and Phillips, 1974
PRO	+	follicle	cow	Walles et al., 1975b
Pro	+	ovary	rabbit	Weiner et al., 1977

[a]Tissue was *in vitro*, except for some rabbit ovaries which were *in vivo*.

ABBR: NE = norepinephrine, E = epinephrine, PE = phenylephrine, TYR = tyramine, IM = imidazole, ISO = isoproterenol, IOS = isoxsuprine, IPA = isoprenaline, TER = terbutaline, POB = phenoxybenzamine, POA = phentolamine, TOL = tolazoline, DB = dibenamine, PIP = piperoxan, PRO = propranolol (NOTE: isoprenaline is a synonym for isoproterenol.)

receptor that is present. If the effector cell contains alpha receptors, then epinephrine is normally the most potent and isoproterenol the least potent, adrenergic agent, with norepinephrine and phenylephrine having intermediate effects. On the other hand, beta receptors are most responsive to isoproterenol and least responsive to epinephrine. In addition, it should be pointed out that alpha and beta receptors can be selectively blocked by specific adrenergic blocking agents (for more detail, see Bahr et al., 1974).

The most common agonist of cholinergic receptors is acetylcholine, along with other agents such as carbamylcholine. Like adrenergic receptors, the cholinergic receptors can also be blocked by a variety of natural and synthetic

Table 2. Effect of cholinergic agents on contractility.

AGENT	EFFECT	TISSUE*	MAMMAL	REFERENCE
AGONIST				
AC	+	ovary	guinea pig	Coutinho et al., 1974
AC	+	ovary	human	Okamura et al., 1974
AC, CAC	+	follicle	sheep	O'Shea and Phillips, 1974
AC	+	ovary	guinea pig	Walles et al., 1974
AC	+	ovary	human, cow	Walles et al., 1974
AC	+	follicle	human	Owman et al., 1975
AC	+	ovary	guinea pig	Maia et al., 1975
AC, BET,O	+	ovary	rabbit	De La Cruz et al., 1976
AC	+	follicle	human	Gimeno et al., 1976
AC, CAC	+	follicle	cow	Walles et al., 1976
ANTAGONISTS				
AT	−	ovary	guinea pig	Coutinho et al., 1974
AT	−	ovary	human	Okamura et al., 1974
AT	−	follicle	sheep	O'Shea and Phillips, 1974
AT	−	ovary	guinea pig	Walles et al., 1974
AT	−	ovary	human, cow	Walles et al., 1974
AT	−	follicle	human	Owman et al., 1975
AT	−	ovary	rabbit	De La Cruz et al., 1976
AT	−	follicle	cow	Walles et al., 1976

*Tissue was *in vitro*, except for some rabbit ovaries which were *in vivo*.
ABBR: AC = acetylcholine, CAC = carbamylcholine, BET = bethanechol, NEO = neostigmine, AT = atropine (NOTE: bethanechol is a synonym for carbamylcholine.)

antagonists.

Effect of neurohumoral agents on contractility. A compendium of the effects of adrenergic and cholinergic agents on the contractility of ovarian tissue is presented in Tables 1 and 2. The different investigations consistently show that ovarian contractility is intensified by agents which stimulate alpha adrenergic and cholinergic receptors. The contractions are inhibited by agents which stimulate beta adrenergic receptors. Each of these responses can be reversed by the application of antagonists which selectively block the specific receptors of the respective neurohumoral agents. (Only Walles et al., 1974, have reported that catecholamines reduce ovarian contractility, however, their records (see Fig. 4 in their report) reveal that epinephrine increases the frequency of rhythmic contractions in the guinea pig ovary and therefore, their data may serve as further evidence that catecholamines stimulate contractility.)

Most investigators have concluded that the experimental data support the hypothesis that ovarian adrenergic (and possibly cholinergic) nerves facilitate ovulation by stimulating the contraction of perifollicular "smooth muscle". However, there is no convincing evidence that nervous excitation of myoid tissue is a crucial event in the ovulatory process. It is true that Virutamasen et al. (1973) have presented indirect evidence to support this hypothesis by reporting that monkey ovaries which were removed during the postovulatory phase of the sexual cycle failed to respond to norepinephrine. But, this is negative datum which, by itself, is insufficient. Besides, to the contrary, O'Shea and Phillips (1974) reported that the strongest contractions in the sheep ovary occurred in luteinized tissue. Furthermore, de la Cruz et al. (1976) were unable to detect any correlation between contractile responses (to cholinergic agents) and the ovulatory period in rabbits. In fact, they concluded that, "if ovarian contractions are involved in the ovulatory process, it would appear that autonomic mediators are not essential for their stimulation."

Nonetheless, it remains evident that neurohumoral agents can effect ovarian contractility and it would be useful to have more specific information about the location of the effector cells for the respective types of nerves. In particular, it would be enlightening to know more about the autonomic innervation of ovarian blood vessels. As Bahr et al. (1974) pointed out, "it is highly probable, since adrenergic nerves are important in vasomotor regulation, that catecholamines may affect the ovulatory process through hemodynamic changes." This possibility deserves investigation. In addition, there may be distinct significance to Fink and Schofield's (1970) observation that "the majority of nerve fibers in the ovary of the cat are distributed to vessels" in the hilum, medulla and around follicles: these same sections of the ovary exhibit the greatest contractility *in vitro* and therefore, blood vessels

may contribute to the contractile responses of ovarian tissue to neurohumoral agents.

Effect of electrical stimulation on contractility. A number of years ago, Kraus (1947) reported that "electrical stimulation, no matter how weak or how strong or in what manner applied, was unsuccessful in either preceptibly contracting the follicle wall or inducing rupture of the mature follicle" in the rabbit. Yet, Okamura et al. (1975) have recently demonstrated that the follicle walls and corpora lutea of human ovaries contract in a dose-dependent way to AC-stimulation. Similarly, Owman et al. (1975) have recorded frequency-dependent contractions in follicle strips from human ovaries (and they concluded this response involved alpha receptors.) In contrast, Weiner et al. (1977) found that electrical stimulation of the ovarian nerve in a perfused ovarian system inhibited contractility in a manner analogous to beta receptor stimulation.

Effect of denervation on ovarian function. Some of the most informative data on the influence of nerves on ovarian contractility and ovulation come from studies involving denervated ovaries. A variety of experiments have involved the grafting of ovaries subcutaneously, intramuscularly or into the anterior chamber of the eye. The results of these implants indicate that "severing the ovary from all neural connection does not interfere with normal ovarian function such as ovulation and steroid secretion" (Bahr et al., 1974). However, Bahr et al. have cautiously pointed out that "when the ovary is grafted to another site, both the vascular and neural components are completely reestablished." Therefore, experimental grafts do not completely resolve the question of whether nerves influence the process of ovulation.

Further clarification on the function of nerves in ovulation has come from the interesting studies by Weiner et al. (1975a,b, 1977). These researchers have demonstrated that unilateral ovarian denervation does not reduce the number of ovulations in the rabbit (Weiner et al., 1975a) nor does denervation prevent pregnancy or reduce the usual number of conceptuses (Weiner et al., 1975b). Confirmation of this evidence that ovarian nerves are not essential for ovulation comes from recent demonstrations that ovulation can even occur in perfused ovaries of humans and rabbits (Stahler et al., 1974; Lambertsen et al., 1976; Hamada et al., 1977). In fact, there were 1.5 times more ovulation points in the perfused rabbit ovaries than in contralateral *in vivo* control ovaries (Lambertsen et al., 1976). At the same time, "no association could be noted between the pattern of ovarian contractions and the likelihood of ovulation or the number of ovulations observed" (Hamada et al., 1977). Thus, now there is unequivocal proof that, if contractile activity is involved in the ovulatory process, neuronal stimulation is not necessary.

Summary. Sympathetic and parasympathetic nerves communicate with effector cells in the hilus, medulla and theca folliculi of the mammalian ovary. Most of the nerves appear to terminate on blood vessels. These nerves may or may not stimulate ovarian contractility. The topical application of alpha adrenergic and cholinergic agents serves to intensify ongoing contractions; whereas, beta adrenergic agents have a relaxing effect on the contractile tissue. Since ovulation and pregnancy can occur in animals with denervated ovaries, it may be concluded that autonomic nerves do not have an essential role in the ovulatory process.

Hormonal Influence on Contractility

In addition to the information on neurohumoral agents, there have been studies on the effects of several other metabolic substances on contractility. Prostaglandin $F_2\alpha$, which increases significantly in ovaries near ovulation, has a consistent record of stimulating ovarian contractility (Virutamasen et al., 1972, 1976; Coutinho et al., 1974; Diaz-Infante et al., 1974, 1975; Okamura et al., 1974; O'Shea and Phillips, 1974; Maia et al., 1975; Gimeno et al., 1976). On the other hand, prostaglandin E_1 (PGE_1) (and usually PGE_2) was either without effect or else decreased contractile activity (Virutamasen et al., 1972, 1973; Coutinho et al., 1974; Okamura et al., 1974; O'Shea and Phillips, 1974; Maia et al., 1975; Gimeno et al., 1976). Since it is well-known that both PGF and PGE increase markedly during the ovulatory process, it is difficult to know whether the net effect of these substances is to stimulate or to inhibit ovarian contractility. LeMaire et al., (1973) have reported a 5-fold increase in the PGF/PGE ratio in rabbit follicles near ovulation and their results suggest that $PGF_2\alpha$ may be dominant during this period.

The application of oxytocin tends to stimulate contractility (Virutamasen et al., 1973, 1976; Coutinho et al., 1974; Roca et al., 1976). However, O'Shea and Phillips (1974) reported negligible or variable responses to this pituitary hormone.

Brief studies with 5-hydroxytryptamine (5-HT) show less consistent results. Gimeno et al. (1976)

report that 5-HT increases ovarian contractility. But, O'Shea and Phillips found that 5-HT (and histamine) caused a relaxation of the tissue.

There are not enough experimental data to allow a conclusion with regard to the influence of steroid hormones on ovarian contractility. Coutinho *et al.* (1974) believe that "estrogens activate and progesterone apparently inhibits ovarian motility." In contrast, Diaz-Infante et al. (1975) have reported that estrogens inhibit and progesterone stimulates ovarian contractility. However, these conclusions are based mostly on inferences, and clarification of the effects of steroid hormones will require more empirical data.

Summary. Prostaglandin $F_2\alpha$ and oxytocin stimulate ovarian contractility, whereas PGE has an inhibitory effect. The influence of steroid hormones and 5-HT are less certain.

CONCLUSION

The pulsation of ovarian tissue is an interesting phenomenon which has led numerous investigators to believe that smooth muscle activity contributes to the process of mammalian ovulation. However, the functional significance of ovarian contractility has not been determined. Some researchers continue to uphold the hypothesis that ovarian contractions force the dissociation and disruption of thecal tissue at the apex of mature follicles (Okamura et al., 1972, 1974, 1975; Bjersing and Cajander, 1974; Virutamasen et al., 1976). But, collagen tunics are tenacious, very tenacious and, it is unlikely that even an extensive amount (much less, an indistinct measure) of myoid tissue could provide the necessary force to implement the mechanical rupture of a mature follicle. Although some follicles may rupture at the moment when an ovarian contraction exerts moderate stress on an enzymically weakened follicle wall, there is still no evidence that contractions are necessary for ovulation.

Others believe that ovarian contractility is involved in the collapse of the follicle and extrusion of the ovum at the time of rupture (O'Shea, 1970, 1971, 1973; Fumagalli et al., 1971; Palti and Freund, 1972; Burden, 1973; Kapinus and Rukosuev, 1975; de la Cruz et al., 1976; Wright et al., 1976; Weiner, 1977). This concept presumes that ovarian smooth muscles give rise to one or more peristaltic-like contractions which begin at the base of a ruptured follicle and push a disloged ovum towards the rupture point. However, there are no experimental data to show that a mature follicle is surrounded by such highly organized myoid tissue. Besides, the follicular antrum in the mammalian ovary is a relatively large cavity, the walls of which would need to collapse against the ovum in order to squeeze it out of the follicle. But, there is no available evidence of a sufficient decrease in the antral volume during the minutes following rupture. It appears more likely that the ovum passively flows out of the follicle with the stream of follicular fluid and plasma transudate which exude form the site of rupture.

Thus, the principal observation of this review is that ovarian contractility is not necessary for ovulation to take place. Nevertheless, ovarian contractions are real and they should have some definable role in ovarian physiology. In this interest, this review closes with the following alternative ideas that might be examined as "working hypotheses" in future studies on the function of ovarian contractility.

It should be kept in mind that ovulation is a "physiologically traumatic" phenomenon in that it involves the rupture and hemorrhage of healthy tissue. Therefore, wound healing and the formation of granulation tissue are necessary steps in the overall process. Under such conditions, "fibroblasts become not only motile but also contractile" (Gabbiani et al., 1973). Thus, myoid-like elements could be present in the ovary for reasons that are unrelated to the forceful displacement of connective tissue or ova.

Secondly, although it is quite a different proposition, it is not unreasonable to consider the possibility that ovarian pulsations act to facilitate the clasping arrangement of the open end of the oviduct around the ovary. In other words, ovarian motion might serve to improve the position of the ostium and increase the efficiency with which it collects ova.

Finally, more attention should be given to a possible relationship between ovarian contractions and circulatory function. As Osvaldo-Decima (1970) suggested some time ago, myoid tissue in the ovary "may be linked with specialized vascular responses." In this regard, it is well-known that capillary permeability increases in follicles near ovulation and it may be that vascular spasms cause a further increase in local circulation in order to minimize cytoplasmic damage during proteolytic degradation of ovulatory tissue.

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